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Rapid Detection of Group B Streptococci Directly from Vaginal Swabs

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Duplicate vaginal swabs were obtained from patients who attended obstetric or gynecologic clinics affiliated with the Magee Womens Hospital in Pittsburgh. One swab was cultured semiquantitatively on 5% sheep blood agar to detect group B streptococci (GBS). The other swab was subjected to a rapid method (25 min) for antigen detection and micronitrous acid exposure to extract the GBS antigen, followed by latex particle agglutination. A total of 464 swabs were evaluated by direct plating. Fifty-two swabs (11.2%) were found to contain GBS. Overall, the rapid method detected 21 of 52, or 40.4%, positive specimens. The sensitivity of the rapid method for identifying the most heavily colonized samples was 85.7%. This method can be used to identify maternity patients who are heavily colonized with GBS and are at high risk of delivering septic infants.

Group B streptococci (GBS) are the major cause of infectious neonatal morbidity and mortality. Maternal vaginal colonization provides the major reservoir of GBS from which neonatal colonization and infection derive during parturition (1, 2). Recent investigations have shown that intrapartum antibiotics administered to mothers with vaginal colonization prevent neonatal colonization and early-onset sepsis with GBS (3, 4, 7, 9; N. Tuppurainen, K. Osterlund, and M. Hallman, Pediatr. Res. **20**:403A, 1986). Effective implementation of this strategy would be facilitated if there was a rapid method of identifying women colonized with GBS just before delivery. This study was undertaken to determine whether methods analogous to those used to rapidly identify group A streptococci directly from pharyngeal swabs (micronitrous acid extraction and latex particle agglutination [LPA]) can be effectively applied to the rapid identification of GBS directly from vaginal swabs.

Duplicate vaginal swabs were collected from women attending private or clinic obstetric and gynecologic offices between March and August of 1986. The specimens were collected by using rayon swabs (Culturette; Marion Scientific Corp., Kansas City, Mo.) and transported to the laboratory. Some swabs were processed immediately, and others were refrigerated at 4°C until processed.

One swab of each pair was inoculated onto a 5% sheep blood agar plate. Each plate was streaked by a semiquantitative method to allow estimation of the density of colonization, which was expressed as 4+, 3+, 2+, and 1+. The designations of 2+, 3+, and 4+ were assigned when at least five colonies of GBS were found on streaks 2, 3, and 4, respectively; a 1+ designation was assigned when GBS growth was limited to the primary zone of inoculation. Women with 3+ or 4+ growth were considered to be heavily colonized, whereas women with 1+ or 2+ growth were considered to be lightly colonized. The plates were incubated aerobically for 18 h in 5% CO_2 at 37°C. Beta-hemolytic streptococcal isolates were classified as B or non-B with Streptex reagents (Wellcome Reagents Div., Burroughs Wellcome Co., Research Triangle Park, N.C.).

The second vaginal swab was placed in a glass test tube (10 by 75 mm). Reagents 1, 2, and 3 from the Bactigen group A streptococcus kit (Wampole Laboratories, Div. Carter-Wallace, Inc., Cranbury, N.J.) were used as directed in the package insert, but the volumes were reduced from 100 to 50 μl each for reagents 1 and 2 and from 200 to 100 μl for reagent 3. After the use of reagent 3, as much fluid as possible was expressed from the swab, and the swab was discarded. The sample was centrifuged in an IEC clinical centrifuge (International Equipment Co., Div. Damon Corp., Needham Heights, Mass.) at the highest speed for 5 min. The supernatant was used as the test fluid in the LPA reaction.

Using the Bactigen kit for group B streptococcus (Wampole Laboratories), we placed 50 μl of the supernatant in each of two wells on a glass serologic slide. This specimen was processed as directed in the package insert, save for the elimination of the specimen-absorbent step, the need for which was obviated by the previous centrifugation procedure. A 10-μl volume of GBS reagent latex was added to one well, and 10 μl of GBS control latex was added to the other. The agglutination reaction pattern was read as positive when dense, white clumps were seen against a clear background; the reactions were always easily interpreted.

To test the sensitivity of this method, 200 μl of decreasing 10-fold dilutions of a 5-h growth of GBS suspension was absorbed by a Culturette swab and extracted. The reagent latex reproducibly gave a positive agglutination reaction with a concentration of 3×10^5 CFU of GBS per ml.

A total of 464 duplicate vaginal swabs were obtained and evaluated. Fifty-two (11.2%) were found to contain GBS by direct plating on 5% sheep blood agar plates. The density of colonization and rate of detection by rapid methods is shown in Table 1. The overall sensitivity of the rapid method to detect GBS colonization was 40.4% (21 of 52). The sensitivity of the rapid method for detecting heavy colonization was 66.7% (18 of 27). Three specimens were found to be falsely positive. The specificity of the rapid method of antigen detection was 99.3% (412 of 415). The predictive value of a positive test was 87.5% (21 of 24). The predictive value of a negative test for any degree of GBS colonization was 93.0%

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TABLE 1. Comparison of density of GBS vaginal colonization and antigen detection by LPA

Density of colonization	No. identified		Sensitivity of +LPA (%)[a]
	Culture	+LPA	
1+	16	2	12.5
2+	9	1	11.1
3+	13	6	46.0
4+	14	12	85.7

[a] A positive LPA reaction.

(412 of 443). The predictive value of a negative test for heavy GBS colonization was 98.0% (437 of 446).

Awareness of the effectiveness of intrapartum antibiotics in preventing neonatal colonization and disease caused by GBS underscores the need for a means of rapid intrapartum identification of parturients who have heavy vaginal colonization with GBS. Timely identification of such individuals would permit selective chemoprophylaxis during labor.

Methods for the rapid identification of group A streptococci directly from pharyngeal swabs have been carefully evaluated regarding sensitivity and specificity. Concern has been raised that these methods may fail to detect from 5 to 40% of colonized patients, most of whom are only lightly colonized (6). Because all these patients are not simply carriers, failure to identify them may have clinical consequences.

In contrast to the need for high sensitivity in identifying individuals who are ill with group A streptococci, methods for the rapid identification of GBS vaginal carriers need only identify the group of women who are heavily colonized. Jones et al. (5) have shown that, almost exclusively, heavily colonized maternity patients delivered heavily colonized infants who, in turn, were at increased risk for developing GBS disease. Antigen detection methods used by Jones et al. (5) and Lim et al. (7) identified percentages of heavily colonized GBS vaginal carriers similar to those which we detected (25, 28, and 40%, respectively). Furthermore, their methods were sufficiently sensitive to identify the mother-infant pairs who were at risk for neonatal GBS sepsis. The advantage of the method of rapid detection described herein is that the results are available within 20 to 30 min of obtaining the vaginal specimen; the procedure does not involve the 5 to 7 h of incubation and processing required by other methods.

Of the 464 swabs, 3 were found to be antigen positive but culture negative. These false-positive reactions may have been caused by other normal vaginal flora which may contain cross-reacting surface antigens or by staphylococci which can cause nonspecific agglutination patterns. Alternatively, unknown antibiotic use by study subjects may have invalidated the culture results.

In two cases of heavy colonization (4+), the rapid method failed to identify the specimen as GBS antigen positive. In each instance, the swab which had been used for the culture was tested for antigen. One was found to be positive, and the other was found to be negative. Since separate swabs were routinely used for culturing and antigen detection, these discordant results may represent sampling error rather than an inadequacy of the antigen detection technique.

Micronitrous acid extraction and LPA can rapidly identify maternity patients with heavy vaginal colonization by GBS who are at high risk of delivering septic infants. Slifkin et al. (8) previously evaluated a similar method using noncommercial extraction reagents and a Phadebact coagglutination technique, but the procedure was cumbersome and not easily adaptable to a clinical laboratory. The ease of performance and interpretation and the rapidity of the test procedure presented here allow timely implementation of intrapartum chemoprophylaxis. The availability of test results in 25 min permits evaluation of those parturients who deliver prematurely (a group which would otherwise elude third-trimester prenatal cultures) or precipitously (a group which would deliver before initiation of chemoprophylaxis based on the results of antigen detection methods requiring a 5- to 7-h period of incubation and processing).

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